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## Isolated Perfusion of Rat Livers: Effect of Temperature on O<sub>2</sub> Consumption, Enzyme Release, Energy Store, and Morphology

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### Abstract

The effect of temperature on isolated rat liver perfusion was studied. Livers were perfused for 12 hours with oxygenated Krebs-Henseleit solution at 5, 10, 15, 20, 25 and 30°C, followed by one hour normothermic reperfusion. After each perfusion, oxygen consumption, liver enzyme release, tissue swelling, energy metabolism and histopathological abnormalities were determined. Compared to the oxygen consumption at 37°C, that of 25, 10 and 5°C was 47%, 16% and 12%, respectively. When the liver was perfused at 30°C, higher enzyme release and lower energy status was observed. Tissue swelling was significant only with livers perfused at 5, 10 and 30°C. After normothermic reperfusion, liver injury indicated by enzyme release and bile production was remarkable with 30°C livers, and that of the other groups was essentially the same as the control. ATP of 5, 25 and 30°C livers was significantly lower than the control. Histopathological examination demonstrated abnormalities of sinusoidal cells and hepatocytes in livers perfused at 25°C and 30°C. Thus higher temperature (25°C to 30°C) during continuous perfusion were found to induce liver damage. Moderate hypothermia between 10°C and 20°C maintained structure and function of rat livers rather well. These results suggest that, when a machine perfusion of the liver is attempted, a wider temperature range, higher than the conventional, needs to be taken into consideration.

### Introduction

The duration of acceptable liver preservation under simple hypothermia has been extended remarkably by the University of Wisconsin (UW) solution<sup>26)</sup>. However, simple hypothermia of the liver can only delay ischemic injury<sup>9,27)</sup>. Consequently, the next major step for truly long-term liver preservation will probably depend upon continuous perfusion techniques<sup>23)</sup>.

The conditions for efficient isolated machine perfusion need clarification for all organs. For the kidneys, the factors studied in the past include constituency of the perfusate<sup>3,4,14)</sup>, pH<sup>6,24)</sup>, perfusion pressure<sup>20,21)</sup>, and its pulsatile versus non-pulsatile characteristics<sup>1,11)</sup>. While the intermediary

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Abbreviations: ADP, adenosine diphosphate; ALT, alanine aminotransferase; AMP, adenosine monophosphate; AST, aspartate aminotransferase; ATP, adenosine triphosphate; LDH, lactate dehydrogenase; TA, total adenine

metabolism of the kidney graft<sup>13,22)</sup> has been the end point for many of these studies, sufficient attention has not been paid to the factor of temperature which usually is controlled as a non-variable at 5–10°C<sup>7,12)</sup>.

For the liver, even this kind of rudimentary information is sparse<sup>5,19)</sup>. A particularly glaring deficit is the lack of data on the role of the perfusion fluid temperature in determining the quality of preservation. Consequently, we have investigated the effect of temperatures between 5 and 30°C of oxygenated Krebs-Henseleit bicarbonate (KHB) solution which was used to perfuse rat livers for 12 hours. Multiple biochemical and morphologic end points were obtained to judge the quality of preservation after the 12 hour preservation and again after the livers were reperfused for one hour with the same solution at 37°C.

### Materials and Methods

#### *Animals:*

Inbred male Lewis rats (Harlan Sprague-Dawley Inc., Indianapolis, IN) weighing 180–270 g were used. All animals were maintained in conventional animal facilities and fed ad libitum with commercial rat chow.

#### *Surgical Procedure:*

The animals were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg, Abbott Laboratories, N. Chicago, IL). Through a cruciate abdominal incision, the common bile duct was cannulated with a 22 G teflon catheter (PE 10, I.D. 0.28 mm, Clay Adams, NJ). Three mg heparin sodium was injected via the penile vein. Through an 18 G teflon catheter inserted into the portal vein, the liver was infused with 60 ml cooled KHB solution while it was decompressed by cutting and cannulating (14 G teflon) the inferior vena cava (IVC) just below the liver. The perfusion pressure was maintained at 10 cm H<sub>2</sub>O. This initial infusion required 4 to 5 minutes. The liver was removed and weighed, before being transferred to the hypothermic perfusion apparatus.

#### *Hypothermic perfusion:*

The extracorporeal system consisted of a perfusion pump (Masterflex, Cole Parmer Instrument Co., Chicago, IL), and oxygenator, a filter, a bubble trapper, inflow and outflow oxygen probes and a reservoir. The oxygenator was a coiled silicone tube (S/P medical grade silicone tubing, Baxter Healthcare Co., Pittsburgh, PA, I.D. 0.147 cm, length 16 m) with an oxygen permeability of 31,500 cc/100 inches<sup>2</sup> which was exposed to a mixture of 95% O<sub>2</sub>-5% CO<sub>2</sub> at a flow rate of 5 L/min. Tygon tubes (R3603 Masterflex, Cole Parmer Instrument Co., Chicago, IL, I.D. 1.6 mm) with an oxygen permeability of 120 cc/100 inches<sup>2</sup> were used as connecting tubes.

The entire apparatus except the pump was placed in an experimental organ preservation system (XOPS-R 10, Olympus Co., Tokyo, Japan) which was designed to control the temperature at any desired level between –15°C and 40°C. The recirculating perfusion medium was 300 ml of KHB solution containing 5.6 mM glucose, 12 mg sulfamethoxazole and 2.4 mg trimethoprim. Because the Clark platinum electrode for oxygen measurement is extremely sensitive to temperature changes, as well as flow rate, the extracorporeal circuit was precirculated for 2 hours before insertion of the liver to be sure that a stable equilibrium had been reached. The flow rate was 3.0–3.5 ml/g liver/min. The pressure which was non-pulsatile ranged from 5 to 10 cm H<sub>2</sub>O.

#### *Normothermic reperfusion:*

After hypothermic perfusion, livers were weighed and transferred into the normothermic perfu-

sion system previously standardized in our laboratory<sup>19)</sup>. Perfusion was continued for one hour after a 15 minute equilibrium time. The perfusion at 37°C was performed with 250 ml of recirculated oxygenated KHB solution at a flow rate of 3.0–3.5 ml/g liver/min.

*Samples:*

Aliquots for analysis of glucose, AST, ALT, and LDH were collected from the reservoir at the end of the 12 hour hypothermic perfusion, or from the outflow line at the end of the one hour normothermic perfusion. For measurement of adenine nucleotides, the left lateral lobe of the liver was clamped with precooled plates and immediately immersed in liquid nitrogen. The median and right lateral lobes were obtained for histopathologic studies and for measurement of water content and sodium and potassium concentration.

*Measurements:*

Oxygen consumption—Oxygen consumption was calculated from the difference in oxygen content of the perfusate entering and leaving the liver, and expressed as  $\mu\text{mol/g liver/min}$ . The oxygen probes and oxymeter used during hypothermic perfusion were those made by the Biochemical Instrument Group of the University of Pennsylvania. A pH-blood gas meter (ABL2 Acid-Base Laboratory, Radiometer, Copenhagen, Denmark) was used for oxygen determination at normothermic reperfusion.

Other perfusate chemistries—AST, ALT, LDH and glucose in the reservoir or outflow line perfusate were determined by a Technicon RA-500 analyzer (Technicon Instrument Co., Tarrytown, NY) using commercially available kits.

Tissue—The wet weight of the whole liver was determined on an analytical balance after uniform blotting with filter paper. The dry weight and tissue water content were determined after drying a weighed portion of the liver in an oven at 105°C for 24 hours and reweighing. Tissue potassium and sodium were measured by a flame atomic emission spectroscopy (FAES, Instrumentation Laboratory Inc., Lexington, MA) after homogenizing the tissue in deionized water.

The separation of adenine nucleotides was performed by single-run high performance liquid chromatography (HPLC) using the modified method of Wynants and van Belle<sup>28)</sup>.

Tissues for histopathological examination were fixed in Bouin's solution for 24 hours, followed by 80% alcohol and stained with hematoxylin-eosin. Histopathologic analyses were done blindly without knowing the timing of tissue collection and experimental groups. Mild to moderate hepatocytic damage was evaluated as irregular arrangements of cords, intracytoplasmic vacuolization and spotty necrosis. Discoloration of hepatocytes with eosinophilic cytoplasm and pyknotic nuclei was graded as moderate to severe damage. Severity of the sinusoidal lining cells was assessed by round or denuded nuclei, cellular detachment from the hepatic cords, or fragmentation.

*Experimental Groups:*

In 42 experiments, the liver oxygen consumption and bile production of fresh livers were measured during one hour of perfusion at temperatures of 5, 10, 15, 20, 25, 30, and 37°C ( $n=6$  at each temperature).

In 36 additional experiments, pump perfusion at these same temperatures (except 37°C) was continued for 12 hours. At the end of these preservation periods, samples were obtained for various metabolic studies of the perfusate and liver as well as anatomic and histopathologic studies of the liver.

Another 36 livers had the same 12 hour hypothermic perfusion ( $n=6$  at each temperature), but the livers were then switched to the normothermic perfusion apparatus and after one further hour of

"reperfusion" at 37°C, the same metabolic and morphologic studies as obtained at the end of 12 hours in the preceding series of experiments were done.

#### Statistics:

All data were presented as mean  $\pm$  standard error of the mean (mean  $\pm$  SEM). Temperature effect on perfused livers was analyzed using an analysis of variance by the statview II software (Abacus Concept Inc., Berkeley, CA). Multiple comparison was performed by student Newman-Keuls test. A p value  $<0.05$  was considered to be significant.

## Results

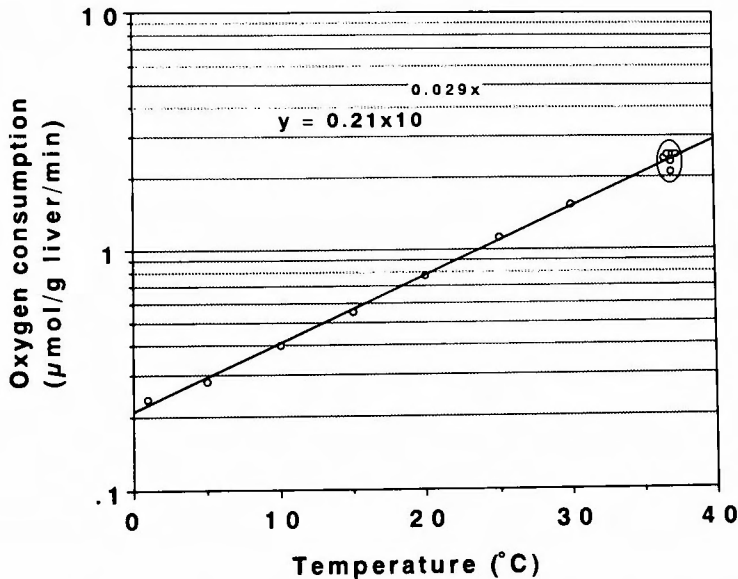
### Non-Preserved Livers

**Oxygen consumption**—There was an exponential decrease in oxygen consumption with reduction in temperature from 37 to 5°C as shown by the straight line slope of an Arrhenius plot (Figure 1). The  $Q_{10}$  was calculated to be 2.0 throughout the temperature range.

**Bile production**—The volume of bile during the one hour perfusion was proportional to the temperature of the perfusate (Figure 2).

### Preserved Livers Before and After Normothermic Reperfusion

**Oxygen consumption**—The O<sub>2</sub> consumption and  $Q_{10}$  at the end of the 12 hour hypothermic perfusion were not different than after the one hour perfusion of fresh livers at these same temperatures (data not shown). When these preserved livers were reperfused at 37°C for one hour, the O<sub>2</sub> consumption was in the same range as with normothermic perfusion of unpreserved livers from the outset for one hour (data shown as "oval" in Figure 1).



**Fig. 1** Oxygen consumption of perfused livers at different temperatures. The mean  $\pm$  SEM of six experiments is given. Ordinate: logarithm of oxygen consumption ( $\mu\text{mol/g liver/min}$ ). Abscissa: reciprocal of absolute temperature.  $Q_{10}=2.0$

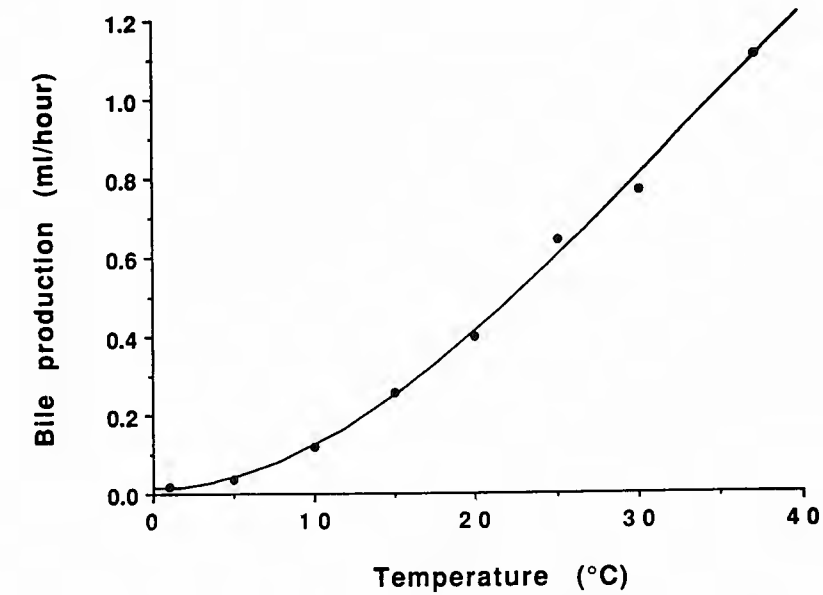


Fig. 2 Effect of temperature on bile production of non-preserved livers during one hour hypothermic perfusion.

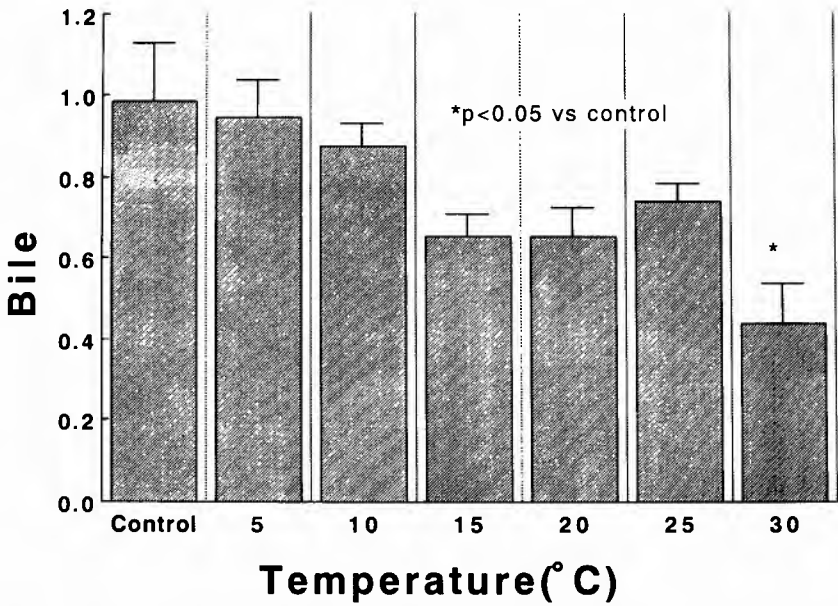


Fig. 3 Effect of temperature on bile production of preserved livers during one hour normothermic reperfusion.

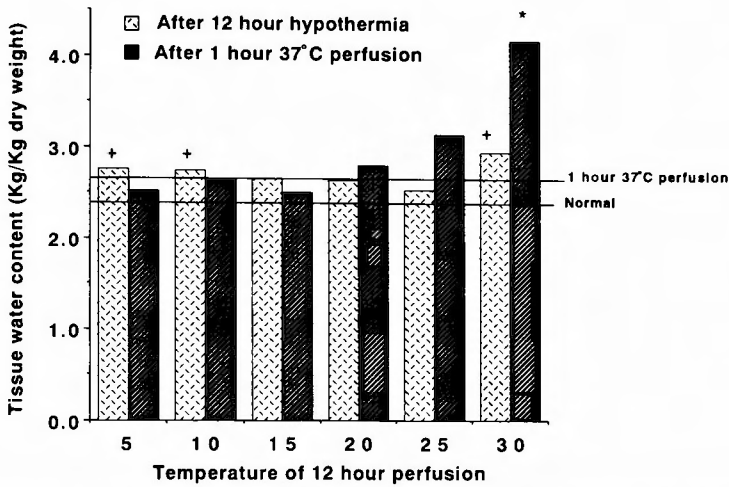


Fig. 4 Tissue water content in livers after 12-hour hypothermic perfusion and after one-hour normothermic reperfusion. +  $p < 0.05$  vs normal fresh livers, \*  $p < 0.05$  vs fresh livers perfused for 1 hour at 37°C.

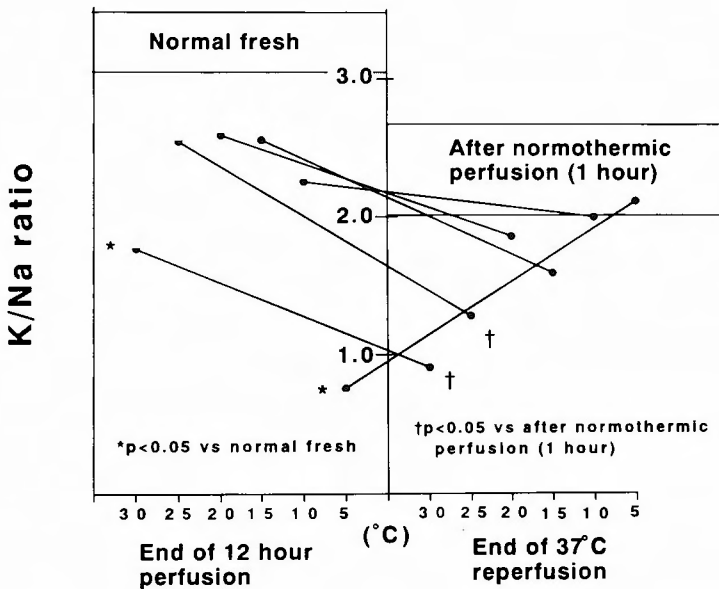


Fig. 5 K/Na ratio in livers after 12-hour hypothermic perfusion and after one-hour normothermic reperfusion.

**Bile production**—Though none of the 12-hour preserved livers produced as much bile during reperfusion at 37°C as the fresh livers which were perfused at this temperature from the outset, there was no significant difference among the temperature groups between 5°C and 25°C. The significant small amount of bile was produced only by the 30°C perfused livers (Figure 3).

**Liver water, sodium, and potassium**—During the 12 hour perfusion, an increase in tissue water

content was seen with all temperatures. This was significant at 5, 10 and 30°C.

After reperfusion (at 37°C), the 12-hour preserved livers in 36 additional experiments, the organs which had been preserved at the temperatures of 30°C only showed significantly increased water content (Figure 4).

The potassium/sodium (K/Na) ratio in 6 normal unpreserved fresh livers averaged  $3.27 \pm 0.22$  (mean  $\pm$  SEM). In other fresh livers, this ratio was reduced to  $2.29 \pm 0.29$  after 60 minutes of perfusion at 37°C (Figure 5). The K/Na ratio was relatively well maintained around 2.3 during 12 hours of hypothermic perfusion at all temperatures except at 5°C where it fell to  $0.75 \pm 0.18$  and at 30°C where it fell to  $1.76 \pm 0.19$ . After normothermic reperfusion, the K/Na ratio in the livers which had been perfused at 5°C was dramatically restored to a normal range. At all other 12-hour perfusion temperatures the K/Na ratio decreased during the subsequent one-hour normothermic reperfusion, however, significant deterioration was only seen in the livers previously perfused at 25 and 30°C (Figure 5).

Liver enzyme release and glucose concentrations—AST, ALT, and LDH were released in small quantities into the recirculated perfusate at all hypothermic perfusion temperatures from 5 to 25°C. There was a large increase of these perfusate enzymes only when the perfusion temperature was 30°C (Figure 6, upper).

Enzyme release as measured in the outflow line from the livers also was minor during the 60

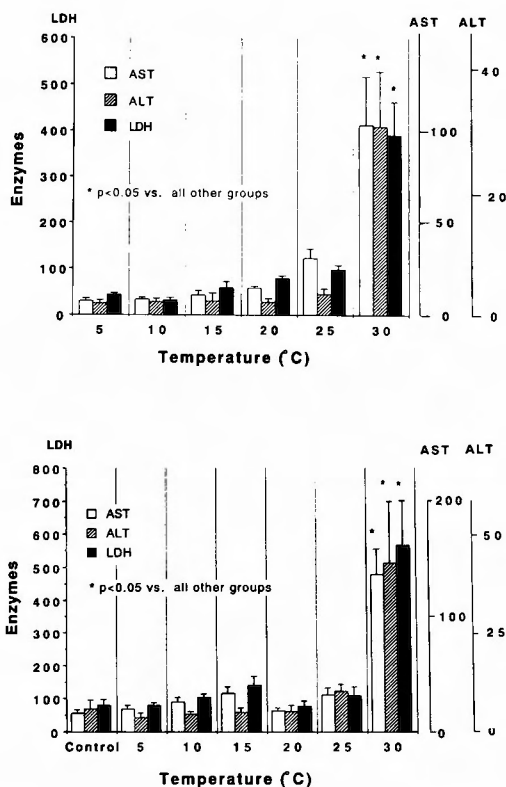
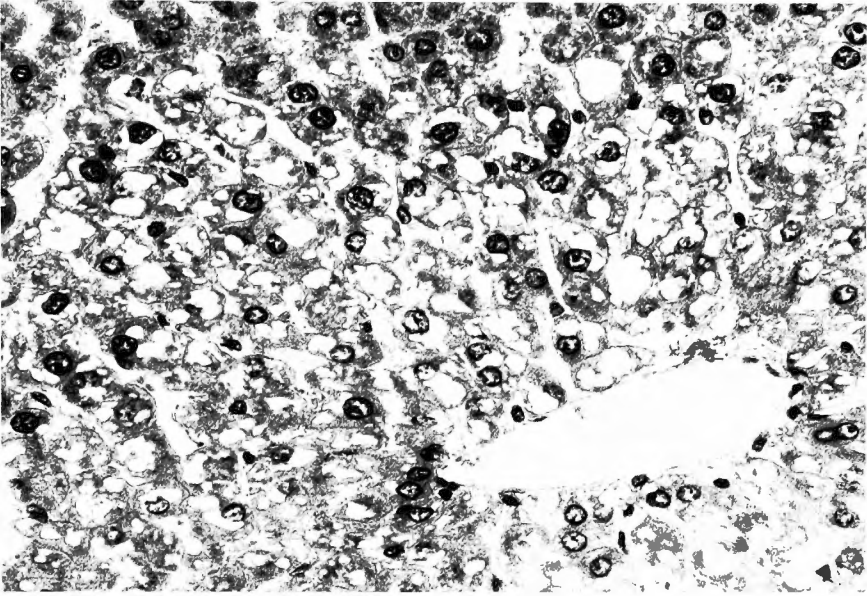
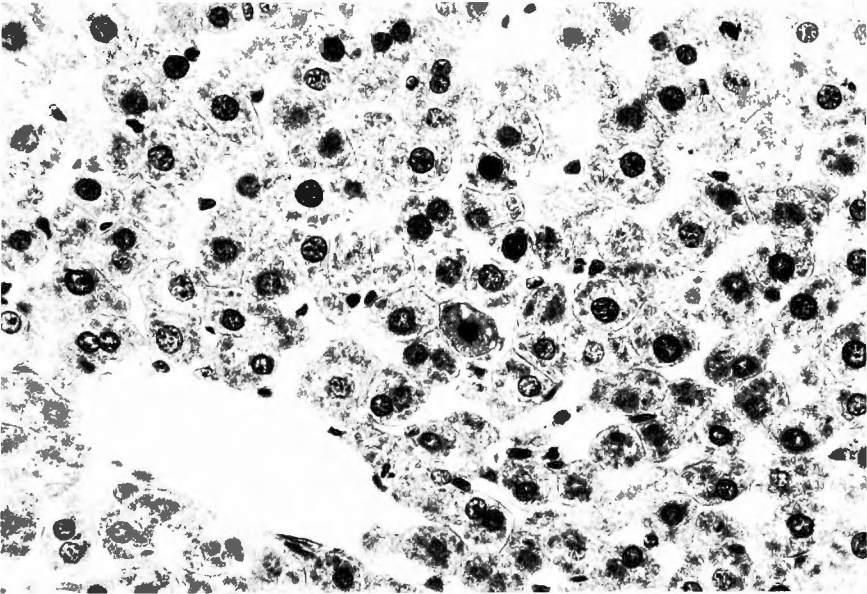


Fig. 6 Upper: Liver enzyme release during 12-hour hypothermic perfusion. Lower: Liver enzyme release during one hour normothermic reperfusion.





**Fig. 7** Liver perfused at 30°C for 12 hours followed by one hour normothermic perfusion. Hepatocytes preferentially in zone 3 strikingly contain cytoplasmic vacuoles varying in size with pyknotic nuclei. Most sinusoidal lining cells are round and detached from underlying hepatocytes. Some sinusoidal spaces contain nuclear debris with the absence of sinusoidal lining cells. ( $\times 40$ )



**Fig. 8** Liver perfused at 20°C for 12 hours followed by one hour normothermic perfusion. Sinusoidal lining cells appear as somewhat rounded profile and attached to the regularly arranged cords of hepatocytes. ( $\times 40$ )

minute reperfusion of the 12-hour preserved livers except when the preparatory 12 hour perfusion had been at 30°C (Figure 6, lower).

Perfusate glucose concentration fell very little from the starting 75–100 mg% range when hypothermic reperfusion was at or below 25°C. However, the glucose declined in the perfusate if the liver perfusion was 30°C (data not shown).

Adenine nucleotides and purine catabolites—The adenine nucleotide profile was well preserved throughout the 12 hour perfusion at all temperatures from 5 to 25°C. Serious adenine nucleotide deterioration during the hypothermic perfusion was seen only at the 30°C temperature (Table 1).

However, at the time of normothermic reperfusion (37°C for one hour) of 12-hour preserved liver, declines in ATP and energy charge with increases in ADP and AMP were seen in separate experiments no matter what the temperature of the prior 12-hour perfusion (Table 1). Severe decreases in total adenine and energy charge were seen when the preceding 12 hour perfusion was at 25 or 30°C (Table 1). These changes reflected the consequences of the 12 hour hypothermic perfusion rather than the normothermic reperfusion which by itself did not drastically change the adenine profile of energy charge of fresh livers (Table 1).

The tissue purine catabolites adenosine, inosin, hypoxanthine, and xanthine were not drastically altered from normal by any of the perfusion procedures.

Histopathologic changes—Histological change of the hepatocytes and sinusoidal lining cells was minimum in livers perfused for 12 hours at 20°C and below, while those perfused at 25°C and 30°C showed mild to moderate damage of these cells. Bacterial contamination was seen in higher temperature groups, 16% (1/6) at 25°C and 67% (4/6) at 30°C.

After the 1 hour normothermic reperfusion, 25°C and 30°C group livers showed moderate to severe histologic damage preferentially at the centrilobular zone, which consisted of contracted

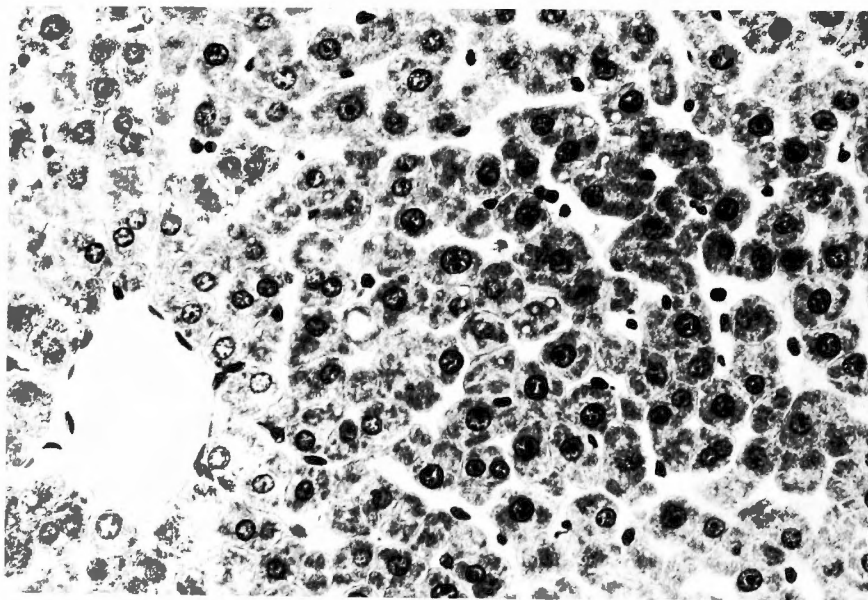


Fig. 9 Liver perfused at 5°C for 12 hours followed by one hour normothermic reperfusion. Most sinusoidal cells show normally elongated appearance lining the regularly arranged cords of hepatocytes. ( $\times 40$ )

**Table 1** Effect of temperature on adenine nucleotides

| Preservation         | ATP                      | ADP                      | AMP                      | TA**                     | EC***                    |
|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <b>Fresh Liver</b>   |                          |                          |                          |                          |                          |
| No perfusion         | 3.55 ± 0.14              | 1.41 ± 0.08              | 0.24 ± 0.03              | 5.21 ± 0.21              | 0.82 ± 0.11              |
| Post-reperfusion*    | 3.52 ± 0.13              | 1.69 ± 0.10              | 0.45 ± 0.09              | 5.67 ± 0.22              | 0.77 ± 0.02              |
| <b>5°C</b>           |                          |                          |                          |                          |                          |
| Post-12h hypothermic | 3.42 ± 0.32              | 1.19 ± 0.18              | 0.22 ± 0.03              | 4.83 ± 0.18              | 0.83 ± 0.03              |
| Post-reperfusion     | 2.33 ± 0.16 <sup>b</sup> | 1.48 ± 0.09              | 0.62 ± 0.11              | 4.43 ± 0.14              | 0.70 ± 0.03              |
| <b>10°C</b>          |                          |                          |                          |                          |                          |
| Post-12h hypothermic | 3.98 ± 0.11              | 1.35 ± 0.08              | 0.18 ± 0.05              | 5.51 ± 0.14              | 0.85 ± 0.01              |
| Post-reperfusion     | 2.84 ± 0.22              | 1.75 ± 0.09              | 1.11 ± 0.58              | 5.71 ± 0.66              | 0.66 ± 0.05              |
| <b>15°C</b>          |                          |                          |                          |                          |                          |
| Post-12h hypothermic | 3.73 ± 0.13              | 1.29 ± 0.06              | 0.15 ± 0.03              | 5.17 ± 0.17              | 0.85 ± 0.01              |
| Post-reperfusion     | 2.69 ± 0.21              | 1.57 ± 0.12              | 0.65 ± 0.06              | 4.49 ± 0.34              | 0.71 ± 0.02              |
| <b>20°C</b>          |                          |                          |                          |                          |                          |
| Post-12h hypothermic | 3.36 ± 0.01              | 1.11 ± 0.03              | 0.12 ± 0.01              | 4.59 ± 0.10              | 0.85 ± 0.01              |
| Post-reperfusion     | 2.69 ± 0.13              | 1.65 ± 0.06              | 0.69 ± 0.09              | 5.04 ± 0.16              | 0.70 ± 0.02              |
| <b>25°C</b>          |                          |                          |                          |                          |                          |
| Post-12h hypothermic | 3.59 ± 0.11              | 1.38 ± 0.11              | 0.15 ± 0.03              | 5.12 ± 0.22              | 0.84 ± 0.01              |
| Post-reperfusion     | 1.92 ± 0.38 <sup>b</sup> | 1.45 ± 0.20              | 0.6 ± 0.13               | 3.97 ± 0.53 <sup>b</sup> | 0.65 ± 0.04              |
| <b>30°C</b>          |                          |                          |                          |                          |                          |
| Post-12h hypothermic | 2.50 ± 0.35 <sup>a</sup> | 1.44 ± 0.09              | 0.36 ± 0.09 <sup>a</sup> | 4.29 ± 0.41 <sup>a</sup> | 0.74 ± 0.03 <sup>a</sup> |
| Post-reperfusion     | 1.19 ± 0.34 <sup>b</sup> | 1.00 ± 0.12 <sup>b</sup> | 0.71 ± 0.12              | 2.69 ± 0.35 <sup>b</sup> | 0.55 ± 0.06 <sup>b</sup> |

\* Reperfusion-60 min perfusion at 37°C

\*\* TA- Total adenine

\*\*\* EC- Energy charge

a p&lt; 0.05 VS non-perfused fresh liver

b p&lt; 0.05 VS post-reperfusion of fresh liver

hepatocytes with eosinophilic cytoplasm and pyknotic nuclei. Sinusoidal lining cells were frequently detached from the hepatocyte cord (Figure 7). These changes were less in livers perfused at 15°C and 20°C (Figure 8), and rare with livers perfused at the lower temperatures (Figure 9).

## Discussion

The reduction in metabolism at reduced temperature is the theoretical justification to use hypothermia both for preservation techniques which do, as well as those which do not, employ continuous perfusion. During static (sluch) preservation of livers at 5°C, oxygen consumption is reduced to 8.2% of that at 37°C<sup>8</sup>). With continuous perfusion, we demonstrated in the present study that oxygen consumption declined exponentially between 37 and 5°C.

However, oxygen consumption is not a measure of organ quality as was demonstrated in this study using multiple parameters of evaluation after perfusion at different levels of hypothermia. Changes in some of the end points suggested that a major adverse event had occurred when the perfusion temperature was brought below 10°C. Below this temperature, the perfused livers imbibed water more than at 10°C or higher. In addition, paralysis of the sodium pump was reflected in a complete reversal of the K/Na ratio. Although no effort was made to limit the consequent potassium extravasation by increasing the potassium concentration in the perfusate, the ratio changes were largely reversible with rewarming by reperfusion.

Under normal physiologic conditions, maintenance of intracellular water is regulated primarily by Na<sup>+</sup>-K<sup>+</sup> ATPase activity and by osmotic and oncotic pressure<sup>7,25</sup>). Although the activity of Na<sup>+</sup>-K<sup>+</sup> ATPase has been described as undetectable when temperatures drop below 20°C<sup>17</sup>), paralysis of this system with reversal of the K/Na ratio and potassium loss could not be detected in our model until the temperatures reached 5°C. Inactivation of the Na<sup>+</sup>-K<sup>+</sup> ATPase enzyme at this low

temperature and consequent disappearance of the electric potential across the plasma membrane<sup>5)</sup> was not a profound cause of tissue edema. Furthermore, when edema did occur, it was very rapidly reversed by the simple expedient of normothermic reperfusion.

Because various measures of the nucleotide pool have been used to assess the quality of preservation<sup>18)</sup> or to predict hepatic graft viability<sup>15)</sup>, the effect of perfusion temperature on the adenine nucleotides and purine catabolites was extensively studied. The biochemical results correlated poorly with the perfusion temperature. Between 5 and 20°C, total adenine pool and energy charge were not drastically depleted during the 12 hour hypothermic perfusion period or during the subsequent normothermic reperfusion. In contrast, livers which were perfused at 25 to 30°C were seriously depleted of total adenines and energy charge after reperfusion.

Our experiments have obvious limitations. The use of a standard non-sanguinous KHB solution is unphysiological. With normothermic KHB reperfusion, the absence of essential substrates would prevent the reconstitution of normal metabolism no matter what the quality of preservation. In addition, in spite of the use of sterile technique and antibiotics, bacterial contamination occurred in the grafts perfused at higher temperature groups (25 and 30°C).

However, the present study still clearly showed that temperatures of 25 and 30°C are not good for liver perfusion and, that the temperatures below 20°C were well tolerable for at least 12-hour isolated perfusion of the liver. When we attempt the immunomodulation or recharge of the graft for future organ preservation, higher metabolic activity, consequently higher temperature, is preferable. With each higher level of perfusion temperature, the metabolic requirements will become more complex as exemplified in our system by the increased uptake and consumption of glucose. To maintain the liver in a warmer state for longer periods, not only glucose but also other substrates such as short chain fatty acids<sup>10)</sup>, amino acids<sup>16)</sup>, or other carbohydrates<sup>2)</sup> will be needed.

Although the isolated perfusion model which we used permits the assessment of individual variables, the ultimate test of transplantation into an intact recipient circulation will be required as the final arbiter of the quality of preservation.

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## 和文抄録

# ラット肝灌流法 (酸素消費, 酵素逸脱, エネルギー貯蔵 および形態における温度の影響)

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移植の為の肝臓保存には保存時間の延長と共に今後の展開として障害肝の Viability の向上, 抗原性修飾が求められており, このためには現在臨床で用いられている Simple cold storage 法にかわって代謝を維持しつつ保存しうる肝灌流法の開発が必須である. 肝の代謝は温度に強く影響されるため, ラット肝を用いて, 肝灌流法における至適温度の研究を行った.

Krebs-Henseleit 液を用いて 5-30度で12時間灌流後, 37度で1時間再灌流を行い, 酸素消費, 胆汁産生量, 組織内水分, 電解質, Adenine nucleotide 量, 灌流液中肝逸脱酵素量, 組織所見を用いて, 評価した.

30度群において, 再灌流後, 胆汁産生量  $0.44 \pm 0.10$  ml/h, 組織 K/Na 比  $0.90 \pm 0.23$  Total adenine 量  $2.89 \pm 0.35$   $\mu$ mol/g, Total adenine 量  $2.89 \pm 0.35$   $\mu$ mol/g, ATP 量  $1.19 \pm 0.34$   $\mu$ mol/g, Total

adenine 量  $2.89 \pm 0.35$   $\mu$ mol/g, Energy Charge  $0.55 \pm 0.06$  と有意に低下し, 灌流液中 AST 量  $136 \pm 22$  IU/L, LDH 量  $566 \pm 136$  IU/L と有意の上昇を示した. 25度群では ATP  $1.92 \pm 0.38$   $\mu$ mol/g, Total adenine 量  $3.97 \pm 0.53$   $\mu$ mol/g, 組織 K/Na 比  $1.27 \pm 0.13$  と有意に低値を示した. 再灌流後の組織所見でも25度群および30度群で, 類洞細胞, 肝細胞の著明な変化が認められた. 5度群では低温灌流時に細胞内カリウムの流出, 水分の取り込みがみられたが, 復温時にこれらの異常は是正された. 10-20度では胆汁産生量, 組織 K/Na 比, Adenine nucleotide profile, 灌流液中肝逸脱酵素量, および組織所見にコントロールと差はなく, 目的に応じて, 20度まで温度範囲をひろげて, 新しい灌流条件の確立を目指すことが可能と考えられた.